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Molecular epidemiological studies on the relationship between indoor coal burning and lung cancer in Xuan Wei, China

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Abstract

The lung cancer mortality rate in Xuan Wei County, China is among the highest in the country and has been associated with exposure to indoor smoky coal emissions that contain high levels of polycylic aromatic hydro carbons. In a case-control study, it was found that the individual susceptibility to lung cancer risk may be increased by GSTM1 null genotype as well as overexpression of the p53 protein. It is likely that the lung cancer excess in Xuan Wei County results from the complex interaction of genetic and environmental factors that will require further research to be understood.

Keywords: GSTM1; Susceptibility; P53 protein expression; Coal emmision; Lung cancer; Indoor air

1. Introduction

The lung cancer mortality rate in Xuan Wei County, China is among the highest in the country (27.7and 25.3 per 100,000 for males and females, respectively) and has been associated with exposure to indoor smoky coal emissions that contain high levels of polycyclic aromatic hydrocarbons (PAHs) (Lan and Robert, 2002). Further, a previous study showed a clear dose-response relationship between benzo(a) pyrene, a carcinogenic and representative member of the PAH family, and lung cancer. As such, the lung cancer experience in Xuan Wei County is both well characterized and relatively unique in that it is caused primarily by a clearly documented environmental exposure (Mumford and He, 1987; He et al., 1991, 1995).

Advances in molecular biology in the early 1990s made possible the incorporation of molecular methods into epidemiology studies. We were able to take advantage of these approaches to learn more about the pathogenesis of smoky coal-induced lung cancer in Xuan Wei County. The primary goals of the project were as follows:

1. To determine if the GSTM1 null genotype, a common genetic polymorphism that results in decreased ability to detoxify PAHs, modifies the smoky coal lung cancer relationship; and
2. to determine if exposure to smoky coal is more strongly associated with lung cancer among cases whose tumor cells abnormally overexpress the p53 protein, which is caused by certain types of mutations in the p53 gene that previously have been linked to PAH exposure.

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2. Methods and materials

In 1994, we designed a population based molecular epidemiology case control study of incident of lung cancer in Xuan Wei County. Between March 1995 and 1996, a total of 122 lung cancer cases that met the study inclusion criteria and 122 individually matched controls were enrolled. A questionnaire was administered by trained interviewers to obtain demographic information, smoking history, and family and personal

Table 1
Distribution of characteristics in lung cancer patients and controls

Variables	Cases ($n = 122$) n (%)	Controls ($n = 122$) n (%)	P*
Sex			
Male	79 (64.8)	79 (64.8)	
Female	43 (35.2)	43 (35.2)	
Fuel type			
Smoky	120 (98.4)	120 (98.4)	
Smokeless	2 (1.6)	2 (1.6)	
Ever smoked (male)			
No	9 (11.4)	9 (11.4)	
Yes	70 (88.6)	70 (88.6)	0.81
Continuous age**	55.11	55.12	0.46
Smoky coal use without ventilation (tons)	172.0–105.6	130.3–77.1	0.001
Pack-years (male)	27.6–20.1	25.8–22.3	0.65

* Based on X^2 test or t test.
** Mean S.D.

Table 2
ORs and 95% CIs for lung cancer according to different factors

	Cases n (%)	Controls n (%)	OR[a](95% CI)	OR[b](95% CI)
GSTM1 values				
Positive	40 (32.8)	62 (49.2)	1.0	
Null	82 (67.2)	60 (50.8)	2.2 (1.3–3.7)	2.3 (2.3–4.2)
GSTT1 values				
Positive	49 (40.2)	58 (47.5)	1.0	
Null	73 (59.8)	64 (52.5)	1.3 (0.8–2.2)	1.3 (0.7–2.3)
Smoky coal use without ventilation (tons)				
<130	51 (41.8)	72 (59.0)	1.0	
≥130	71 (58.2)	50 (41.0)	2.4 (1.3–4.4)	2.4 (1.3–4.4)
Pack-years (PY)[c]				
0<PY<25	27 (38.6)	36 (52.2)	1.0	
PY≥25	43 (61.4)	33 (47.8)	1.7 (0.9–3.1)	1.5 (0.8–2.9)
COPD				
No	76 (62.3)	87 (71.3)	1.0	
Yes	46 (37.7)	35 (28.7)	1.5 (0.9–2.5)	1.3 (0.7–2.2)
Family history of lung cancer				
No	102 (83.6)	108 (88.5)	1.0	
Yes	20 (16.4)	14 (11.5)	1.6 (0.7–3.5)	1.6 (0.6–3.8)

[a] OR and 95% CIs obtain variate conditional logistic regression.
[b] OR and 95% CIs adjusted for total smoky coal use without ventilation, packyears, COPD, and family history of lung cancer by multiple condition logistic regression.
[c] Evaluated only in male smokers by unconditional logistic regression.

medical history. In addition, a series of questions were asked to estimate each subject's lifetime cumulative household exposure to smoky coal combustion products. Buccal cell and sputum samples were collected from each subject. DNA was extracted from buccal cell samples and analyzed by PCR-RFLP for the presence of the GSTM1 and GSTT1 null genotypes. Sputum samples were fixed in Saccomanno solution and stained by the Papanicolaou method to identify tumor cells. A total of 97 cases had tumor cells detected in their sputum. Samples from these cases and their matched controls (all of whom had no detectable tumor cells) were stained by immunohistochemistry to identify cells that overexpressed the p53 protein, referred to as p53 positive samples (Lan et al., 2000, 2001).

3. Results

Table 1 shows the general characteristics of the cases and controls in the GST study. There were no significant differences in sex, age, fuel type and cigarette smoking.

Table 2 shows the ORs and 95% CIs for lung cancer according to various factors. It was found that the GSTM1 null genotype was associated with a 2.3-fold (95% CI = 1.3–4.2) increased risk of lung cancer. When smoky coal exposure was analyzed as a continuous variable and risk per unit dose estimated from the regression equation, the risk of lung cancer was increased by 1.7-fold per 100 tons (95% CI = 1.3–2.4) for all study subjects, by 1.2-fold per 100 tons (95% CI = 0.8–1.9) among GSTM1 positive subjects, and by 2.4-fold per 100 tons (95% CI = 1.6–3.9) among subjects with the GSTM1 null genotype (test for multiplicative interaction, $P = 0.05$). In contrast, the GSTT1 null genotype was not significantly associated with lung cancer risk, which was expected given that GSTT1 is not thought to metabolize PAHs.

Table 3 presents the general characteristics of the cases and controls in the p53 study. No significant differences were found in the various characteristics between the cases and controls as well as between the p53 positive cases and the p53 negative cases. The results in Table 4 show that smoky coal exposure was more strongly associated with risk of p53 positive than for p53 negative lung cancer. There was some evidence in women, almost all of whom were nonsmokers, that very high levels of smoky coal exposure (i.e., >240 cumulative tons)were strongly and statistically significantly associated with risk of p53 positive lung cancer (OR: 18.72, 95% CI = 1.77–383.38). In contrast, high levels of smoky coal exposure were less strongly associated with p53 negative lung cancer and the association was not statistically significant (OR: 4.80, 95% CI = 0.66–43.87, respectively).

Table 3
Distribution of characteristics in control subjects and lung cancer cases

	Controls		Cases			
			p53 positive ($n = 44$)		p53 negative ($n = 53$)	
	n	%	*n*	%	*n*	%
Age						
<55	42	43	16	36	25	47
>55	55	57	28	64	28	53
Pack-years[a]						
<20	27	44	8	24	13	46
>20	34	56	25	76	15	54
Smoky coal[b](tons)						
Total						
<130	57	59	20	45	23	43
130–240	31	32	12	27	21	40
>240	9	9	12	27	9	9
Male						
<130	36	59	17	52	14	50
120–240	19	31	10	30	10	36
>f240	6	10	6	18	4	14
Female						
<130	21	58	3	27	9	36
130–240	12	33	2	18	11	44
>240	3	18	6	55	5	20

[a] Male participants only.
[b] Smoky coal use without ventilation.

We also looked at the impact of the GSTM1 null genotype for risk of p53 positive and p53 negative lung cancer. Among all 97 cases with tumor cells present in their sputum samples, the GSTM1 null genotype was associated with a 2.2-fold risk of lung cancer (95% CI = 1.1–4.2). There was a greater effect of the GSTM1 null genotype among the p53 positive cases (OR: 2.8, 95% CI = 1.2–6.5), which was statistically significant, whereas the effect was weaker and not significant among the p53 negative cases (OR: 1.6, 95% CI = 0.8–3.3).

Table 4
Multivariate adjusted ORs and 95% CIs for p53+ and p53− lung cancer in Xuanwe, China

	Combined P53+ and p53−		P53+		P53−	
	OR	95% CI	OR	95% CI	OR	95% CI
Smoky coal[a](tons)						
Total						
<130	1.0		1.0		1.0	
130–240	1.48	0.73–3.20	1.08	0.40–2.85	1.63	0.73–0.64
>240	3.21	1.23–9.03	3.98	1.23–13.65	2.39	0.74–7.80
P for trend	0.01		0.03		0.07	
Female						
<130	1.0		1.0		1.0	
130–240	2.21	0.64–8.14	1.49	0.10–17.22	2.47	0.66–10.19
>240	7.94	1.46–60.44	18.72	1.77–383.38	4.80	0.66–43.87
P for trend	0.008		0.007		0.07	
Male						
<130	1.0		1.0		1.0	
130–240	1.30	0.50–3.15	1.01	0.32–3.13	1.53	0.47–43.98
>240	1.88	0.54–7.09	2.01	0.44–9.27	1.81	0.32–9.37
P for trend	0.32		0.47		0.41	
Pack-years[b]						
<20	1.0		1.0		1.0	
≥20	1.57	0.66–3.81	2.51	0.82–8.39	0.99	0.33–3.01

[a] Adjusted for age and gender and smoky coal use without ventilation.
[b] Adjusted for age and gender and pack-years of cigaratte.

For detail results please refer to Lan et al. (2000, 2001).

4. Discussion

This is the first study to provide evidence that the GSTM1 null genotype is associated with increased risk of lung cancer in Xuan Wei County and that it interacts with smoky coal emissions. Also, it is one of the only examples in the literature of an interaction between a true environmental exposure and genetic susceptibility for cancer risk. Given that GSTM1 plays an important role in detoxifying PAHs such as benzo(a)pyrene, our finding lends weight to the hypothesis that PAHs contribute to the pathogeneses of lung cancer in Xuan Wei County. Further, because p53 overexpression has previously been linked to PAHs exposure (Bennett et al., 1991; Iggo et al., 1990), our finding of a very strong association between smoky coal use and p53 overexpression, and a stronger and significant impact of the GSTM1 null genotype on p53 positive samples, lends further support for PAHs being causative agents in the Xuan Wei cancer excess. Therefore, in addition to the basic scientific value of our observations, they also provide support for the benzo(a)pyrene regulations that have been developed based on the lung cancer excess in Xuan Wei County. An important followup study to this project would be to collect tumor samples from lung cancer cases in Xuan Wei to carry out mutation analysis of the p53 gene at the DNA level, which may provide even more specific evidence of a role for PAH exposure.

Secondly, the observation that the GSTM1 null genotype is associated with lung cancer in this population provides support for further study of polymorphism in other genes involved in the activation of PAHs, and in the repair of DNA damage due to PAH exposure, It is likely that additional genetic risk factors will be identified in this population, and that along with the GSTM1 null genotype, will identify highly susceptible subgroups who may benefit from more intensive lung cancer screening efforts.

In summary, smoky coal emissions are etiologically important to the excess of lung cancer in Xuan Wei County. The GSTM1 null genotype may enhance susceptibility to lung cancer due to these indoor coal combustion emissions. Smoky coal use was strongly associated with overexpression of p53 in tumor cells among highly exposed women. It is likely that the lung cancer excess in Xuan Wei County results from the complex interaction of genetic and environmental factors that will require further research to understand.

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